REMARKS

Claim 28 has been canceled and claim 27 has been amended to incorporate the limitations of claim 28, now canceled, and to recite a "promoter". This amendment is supported throughout the application as filed, e.g., at page 14, lines 5-13. Claims 31, 64, 68, 70, 73, 74 and 82 have been amended merely to correct claim dependencies. Claims 27, 30-31, 60-61, 64-74, 79-83 and 86 are pending and under examination. No new matter has been added.

The Invention

The claims are directed to method of producing insulin in a subject in vivo. The method includes introducing into the subject an intermediate lobe (IL) pituitary cell that has been genetically engineered to express insulin. It was found that IL cells (which are regulated secretory cells) have the proper prohormone processing machinery to produce and secrete fully processed, mature insulin sufficient to produce a therapeutic effect in a diabetic subject even when expressed under the control of a promoter that is not glucose sensitive, e.g., a POMC promoter. Importantly, it was found that IL cells are able to evade host immune attack, even in a setting of active autoimmunity.

Claim Objections

The numbering of the claims is objected to as there were two claims numbered 66. Applicants apologize for the error. The claims have been renumbered and dependencies have been corrected as appropriate, as requested by the Examiner.

Rejections Under 35 U.S.C. §112

Written Description

All the pending claims are rejected as "containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." The Examiner argues as follows:

> the specification only discloses a single promoter that is active in intermediate lobe pituitary cells, i.e., the pro-opiomelanocortin (POMC) promoter . . . .the specification does not describe a representative number of species of promoters that are active in IL cells. Thus, one of skill in the art could not envision the entire genus of promoters that are active in IL cells and consequently, the written description requirement has not been met. (Page 4 of the Office Action).

The Examiner concludes that "the written description requirement is not satisfied for the genus of heterologous control regions recited in the claims." This rejection has been met, in part, and is traversed, in part. Claim 27 has been amended to recite a promoter that directs expression in an IL pituitary cell. Support for this amendment can be found in the specification, e.g., at page 14, lines 5-13, which provides as follows:

> Recombinant genes should be placed under the control of a control region which directs sufficient expression of the gene in the intermediate lobe pituitary cells. Tissue specific promoters can be used. Tissue specific promoters, that is promoters which express in the immunologically privileged cells but not in some or all other cell types, may be more desirable where a transgenic animal is used to supply genetically engineered tissue, as the genes will not be expressed in all cells of the transgenic animal. The POMC promoter is particularly suited for use with intermediate lobe pituitary cells. Constitutive promoters can be used. Constitutive promoters include viral promoters, for example, cytomegalovirus promoters, and SV-40 early gene promoters. (Page 14, lines 5-13, emphasis added.)

As can be seen by the above-quoted passage, the choice of promoter that can be used in the claimed methods is not limiting and includes IL-specific promoters and constitutive promoters. The Examiner's statement that "the specification *only discloses a single promoter* that is active in intermediate lobe pituitary cells, i.e., the pro-opiomelanocortin (POMC) promoter" (emphasis added) is simply not true. As can be seen by the above-quoted passage, in addition to the POMC promoter, the specification discloses a representative number of constitutive promoters (e.g., CMV and SV-40 early promoters) that can be readily used in the claimed methods. See also page 27, lines 29-32, of the specification, showing that CMV promoter directs expression in IL cells. As discussed in the enclosed declaration of Dr. Myra Lipes under 37 C.F.R.§ 1.132 (hereinafter "the Lipes declaration"), other constitutive promoters that were routinely used in the art at the time of filing include the JC polymovirus promoter, and

the chicken beta-actin promoter. Using constitutive promoters to drive expression in mammalian cells was routine in the art at the time of filing. In addition, IL-specific promoters besides POMC promoter were known, such as the prodynorphin (proDyn) promoter. See, e.g., Naranjo et al. (1991, Neuron 6(4):607-17), describing the proDyn promoter, and Day et al. (1993, Endocrinology 133:2652-2659), describing proDyn gene expression in IL cells.

A written description inquiry must be made from the standpoint of one having ordinary skill and knowledge of the art. The *Guidelines for the Examination of Patent Applications Under the 35 U.S.C. §112, paragraph 1 "Written Description" Requirement* (Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday, January 5, 2001) (hereinafter "the Guidelines") provide that "[t]he absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. § 112, paragraph 1, for lack of adequate written description" (emphasis added). The Guidelines also state that "what constitutes a 'representative number' is an inverse function of the skill and knowledge of the art." In the present case, the skill and knowledge of the art necessary to select a promoter to express a protein in any one particular cell type was extremely high. It was a routine and established procedure in the art at the time of filing to use, e.g., a constitutive promoter or a promoter specific to the relevant cell type. As discussed above, both constitutive promoters and promoters specific to IL cells were known in the art. The invention does not lie in the choice of promoter (which was routine) but in the choice of IL cells. Thus, a skilled artisan would have understood from the disclosure of the representative CMV, SV-40 early, and POMC promoters that Applicants were in possession of the entire genus of promoters that can drive expression in IL cells. In view of the level of knowledge and skill in the art at the time of priority, disclosure of three species of promoter that can direct expression in IL cells (including 2 constitutive and 1 IL-specific promoter) is representative of the entire genus and is sufficient to show possession of the entire genus. Accordingly, the written description requirement is satisfied.

Enablement

All the pending claims are rejected for lack of enablement. The Examiner asserts that: "The specification fails to provide an enabling disclosure for the use of transgene constructs that

do not encode insulin or do not include the POMC promoter because the proper regulation of insulin secretion is critical for successfully carrying out the claimed method." This rejection has been met, in part, and is traversed, in part.

Claim 27 has been amended to recite that the IL cell includes a nucleic acid sequence encoding insulin, thereby obviating the Examiner's first basis for the rejection. The Examiner's second basis for the rejection, that the claimed methods are only enabled for use of the POMC promoter, are traversed.

Applicants have shown that IL cells have the proper prohormone processing machinery to produce and secrete fully processed, mature insulin sufficient to produce a therapeutic effect in a diabetic subject. See section bridging pages 25 and 26 of the specification, as follows:

> The ability of the transgenic intermediate lobe pituitary cells to efficiently process and secrete mature insulin, along with their resistance to autoimmune attack and injury, show that these cells can be used as a vehicle for insulin replacement in IDDM. Indeed, it was found that transplantation of 4 intermediate lobe pituitaries under the kidney capsule of spontaneously diabetic NOD mice resulted in a significant gain in body weight (Fig 3A) and in the complete remission from diabetic symptoms. This was associated with the progressive return to near-normoglycemia (Fig 3B), with mean BG levels decreasing from 484 $\pm$ 21 mg/dl pre-transplantation to 150 $\pm$ 43 mg/dl after transplantation (n=6). In parallel with this drop in BG, random insulin levels increased from 4 + 0.2 μU/ml pre-transplantation to 42 + 9 μU/ml post-transplantation, in a similar range to random insulin levels of nondiabetic control mice [39 + 9 μU/ml (n=6)]. At the end of the transplantation period, immunohistochemistry of the grafts of the recipients showed abundant insulin staining with no evidence of lymphocytic infiltration. Similar analysis of pancreatic sections from recipients did not reveal any significant insulin-positive cells, confirming that the enhanced insulin levels post-transplantation were due to the transgenic tissue implants. These results demonstrate that the intermediate pituitary-derived insulin is biologically active and are consistent with the biochemical studies which showed that the great majority of insulin secreted by the transgenic pituitaries is fully processed, mature insulin. Diabetic NOD mice receiving nontransgenic (control) intermediate lobe pituitaries had no reduction in serum BG levels, and had increasingly severe diabetic symptoms which resulted in their demise within 3 weeks after transplantation.

As discussed in the Lipes declaration, this experiment was done using an exemplary promoter, the POMC promoter, **which is not glucose sensitive** in the premier animal model for

diabetes, the NOD mouse. Thus, the specification shows that the claimed methods work even in the absence of insulin secretion being tightly coupled to serum glucose concentrations. There is absolutely no reason to think (and the Examiner has provided no evidence) that other promoters, including other IL-specific promoters or constitutive promoters, would not work as well.

The references cited by the Examiner express the idea that the "ideal" surrogate cell for expressing insulin will have tight coupling of insulin secretion to serum glucose levels to avoid the potential for episodes of hypoglycemia or hyperglycemia. As discussed in the Lipes declaration, Applicants do not disagree that the "ideal" or "perfect" insulin secreting cell would be glucose-sensitive. However, this does not mean that the claimed methods are not enabled. Enablement for a method of treatment does not require that there be absolutely no side effects, or that a treatment be perfect or complete. It is difficult to envision any method of treatment of any disorder that would be patentable if that were the standard. Indeed, the standard treatment for diabetes, the self-administration of exogenous recombinant insulin, is obviously not free of risk of episodes of hypoglycemia or hyperglycemia. Furthermore, even treatment with basal levels of insulin can be therapeutic. Therefore, the cited references do not show non-enablement of the claimed methods. The claimed methods work to provide insulin in vivo to a subject, sufficient to cause a therapeutic effect. The claimed methods, as shown by the disclosed use of the POMC promoter, are not dependent on the use of a glucose sensitive promoter. Therefore, the full scope of the claims is enabled.

In addition, the references cited by the Examiner acknowledge that another characteristic of an "ideal" surrogate insulin is the ability to evade the host immune system. The Halban abstract states that "it will be necessary to ensure that newly created or implanted (surrogate) β-cells are protected in some way from recognition by the immune system and in particular from autoimmune destruction." Welsh states that "transplantation of human or pig islets to diabetic recipients is problematic due to poor grafting and rejection" (page 181). Importantly, the claimed methods address precisely this issue given that the IL cells can evade the host immune system.

In light of the foregoing, Applicants respectfully request that the rejections be withdrawn.

Attorney's Docket No.: 10276-015002

Enclosed is a Petition for Extension of Time along with the required fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 9 Apr 2004

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